Proposal Reviews

#221: Identifying the cause of toxicity in environmental samples through gene expression fingerprinting

US Army Corps of Engineers

Initial Selection Panel Review	
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Prior Performance/Next Phase Funding	
Environmental Compliance	
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Initial Selection Panel Review:

CALFED Bay-Delta 2002 ERP PSP
Initial Selection Panel Review

Proposal Number: 221

Applicant Organization: US Army Corps of Engineers

Proposal Title: Identifying the cause of toxicity in environmental samples through gene expression fingerprinting

Please provide an overall evaluation rating.

Explanation of Recommendation Categories: Fund

- **As Is** (a proposal recommended for funding as proposed)
- **In Part** (a proposal for which partial funding is recommended for selected project phases or components)
- **With Conditions** (a proposal for which funds are recommended if the applicant contractually agrees to meet the specified conditions)

Consider as Directed Action in Annual Workplan (a proposal addressing a high priority action that requires some revision followed by additional review prior to being recommended for funding)
Not Recommended (a proposal not currently recommended for funding-after revision may be considered in the future)

Note on "Amount":

For proposals recommended as Fund As Is, Fund In Part or Fund With Conditions, the dollar amount is the amount recommended by the Selection Panel.

For proposals recommended as Consider as Directed Action in Annual Workplan, the dollar amount is the amount requested by the applicant(s).

Fund	
As Is	**-**
In Part	**-**
With Conditions	**-**
Consider as Directed Action	**-**
Not Recommended	**X**

Amount: **$0**

Conditions, if any, of approval (if there are no conditions, please put "None"):

none

Provide a brief explanation of your rating:

This project addresses the need to develop improved methods for Toxicity Identification Evaluation methods by using molecular biological techniques. As a research proposal this project was judged to have significant potential to improve the basis for understanding the complex issue of what compounds are specifically causing toxicity in the environment. The major drawbacks are that the project has a significant level of risk and may take years before real benefits are accrued by CALFED ecosystem restoration efforts. The major decision with this proposal is whether CALFED chooses to fund the development of techniques that may be useful down the line, or apply established techniques now and wait for better methods to be developed by more mainstream funding sources for toxicological studies, such as EPA. The risks appear be too high and the near term benefits too low for CALFED to fund this now.

Research and Restoration Technical Panel Review:

CALFED Bay-Delta 2002 ERP PSP
Research and Restoration Technical Panel Review Form

Proposal Number: 221

Applicant Organization: US Army Corps of Engineers

Proposal Title: Identifying the cause of toxicity in environmental samples through gene expression fingerprinting

Review:

Please provide an overall evaluation summary rating:

<u>Superior:</u> outstanding in all respects;
<u>Above Average:</u> Quality proposal, medium or high regional value, and no significant administrative concerns;
<u>Adequate:</u> No serious deficiencies, no significant regional impediments, and no significant administrative concerns;
<u>Not Recommended:</u> Serious deficiencies, significant regional impediments or significant administrative concerns.

Overall Evaluation Summary Rating	**Provide a brief explanation of your summary rating**
-Superior	**This is an exciting and novel project that offers a promising alternative to traditional TIE techniques. However there are some deficiencies raised by the external reviews.**
XAbove average	
-Adequate	
-Not recommended	

1. **<u>Goals and Justification.</u>** Does the proposal present a clear statement of goals, objectives and hypotheses? Does the proposal present a clear justification and conceptual model for the project?

   Goals, objectives and hypotheses are clearly stated and justified.The hypothesis for the work is that exposure of an organism to a toxic substance will evoke a compound-specific pattern of gene transcription as the organism responds to the toxicant by synthesizing certain proteins. This application of molecular methods to addressing ecotoxicological problems is both timely and novel.

   Justification for the project is provided by evidence for problems with unexplained toxicity that could not be addressed (for one reason or another) with traditional TIE methods. The applicant also documents the disadvantages of current TIE approaches, including high cost, lack of methods for certain key chemicals (e.g., pyrethroids) and poor success with certain media (i.e., sediment). The conceptual diagram is very clear.

2. **Likelihood of Success (Approach, Feasibility, Capabilities and Performance Measures).** Is the project likely to succeed based on the approach, feasibility and project team capabilities? Are the proposed performance measures adequate for measuring the project's success?

   The approach is in general well designed and appropriate for meeting the projects objectives. The approach is fully documented and appears to be technically feasible, however some limitations and problems with the details were highlighted. Several aspects of the approach make it either difficult to evaluate the chance of success or reduce it. Limitations in the number of toxicants, interactions and concentrations included in the development of the microarray are unavoidable, but a modeling (e.g., power analysis) of the required library size would have reduced the uncertainties. Also the evaluation step of the project appears to lack a thorough and unbiased analysis of the success of the methodology in performing the TIEs.

   Generally no issues with regard to capabilities. Though one reviewer questioned what Don Westons contribution to the project would be (i.e. no experience in molecular genetics) yet he receives a large fraction of the budget.

3. **Outcomes and Products.** Will the project advance the state of scientific knowledge in general and/or make an important contribution to the state of knowledge of the Bay-Delta Watershed? For restoration proposals, is the project likely to contribute to ecosystem restoration or species recoveries in a significant way? Will the project produce products useful to decision-makers and scientists?

   Potentially a very valuable outcome would be the demonstration that this approach (gene expression fingerprinting) can be used for identifying the cause of toxicity in environmental samples.

4. **Cost/Benefit Comments.** Is the budget reasonable and adequate for the work proposed?

   This 3 year project has a total budget of $1,093,337. One reviewer raised some questions as to the division of tasks. No salary appears to be requested for the statistician (who will play an essential role in the project). Possibly excessive funds for graduate student salary ($3373/mo), which is higher than the post-docs ($3227). The panel could not understand why such a large fraction of the budget was going through ACOE, Vicksburg.

5. **Regional Review.** How did the regional panel(s) rank the proposal (High, Medium, Low)? Did the regional panel(s) identify significant benefits (regional priorities, linkages with other activities, local involvement) or impediments (local constraints, conflicts with other activities, lack of local involvement) to this proposal? What were they?

   Bay Regional Review rates project as medium because it delivers scientific information which improves understanding about key ecosystem processes in the Bay and Suisun Marsh

   Delta Regional Review rates project as medium because it is feasible and uses leading edge technology, and risk of failure acknowledged by applicant. Sacramento Regional Review ranks the proposal low because it is basic research on developing new test protocols for determining the sources of toxicity and ERP benefits, if any will be a long time in coming.

   San Joaquin Regional review ranks the proposal low because it takes a shot-gun approach to the search for causes of unknown toxicity and considers that more experimental aspects should be understood and demonstrated before it is applied in this context.

6. **<u>Administrative Review.</u>** Were there significant concerns about the proposal with regard to the prior performance, environmental compliance and budget administrative reviews? What were they?

   Re. Prior Performance: NFWF does not have any direct agreements with US Army Core of Engineers. Current recipient for 99-N08 is UC Berkeley. UC Berkeley listed as subcontractor on this project. Prior performance satisfactory.

   Re. Environmental Compliance: no issues.

   Re. Budget: need to verify that OH rate for UCB fluctuates with funding source since all funds will flow through a federal applicant. Cost share of $300K/yr is indicated but proposal does not say how these funds will be applied to project.

Miscellaneous comments:

None

Bay Regional Review:

Proposal Number: 221

Applicant Organization: US Army Corps of Engineers

Proposal Title: Identifying the cause of toxicity in environmental samples through gene expression fingerprinting

Overall Ranking: **-**Low **X**Medium **-**High

Provide a brief summary explanation of the committee's ranking:

The panel supports research that delivers scientific information which improves understanding about key ecosystem processes in the Bay + Suisun Marsh or about species and habitats which are insufficiently understood.

1. Is the project feasible based on local constraints?

   XYes **-**No

   How?

   Yes, this project is feasible.

2. Does the project pursue the restoration priorities applicable to the region as outlined in the PSP?

   XYes **-**No

   How?

   MR #5 ensure restoration not threatened by degraded environmental water quality

3. Is the project adequately linked with other restoration activities in the region, such as ongoing implementation projects and regional planning efforts?

   XYes **-**No

   How?

   the project has close ties to the project described in the proposal submitted by SFEI

4. Does the project adequately involve local people and institutions?

   XYes **-**No

   How?

Yes the project has close ties to the project described in the proposal submitted by SFEI, and which will be providing environmental samples to this effort and collaborating closely.

Other Comments:

At this time, the regional panel favors environmental water quality projects that provide information about a particular known toxic pollutant (mercury)in the region, especially when the information can be helpful in making decisions about restoration projects.

Delta Regional Review:

Proposal Number: 221

Proposal Title: Identifying the cause of toxicity in environmental samples through gene expression fingerprinting

Overall Ranking: **-**Low **X**Medium **-**High

Provide a brief summary explanation of the committee's ranking:

Though this project appears feasible, this is leading edge technology with a risk on failure acknowledged by the investigator.

1. Is the project feasible based on local constraints?

   XYes **-**No

   How?

   This is mostly a lab experiment that will be using chironomids collected either by UC Berkeley or the principal investigator. The applicants make it clear that this is a new technology with no guarantee of success. The work is planned to accompany an additional proposal submitted to CALFED by SFEI, however, the two proposals are mutually exclusive and can continue regardless of the other's fate. This technique has been very successful in determining types of cancer in humans.

2. Does the project pursue the restoration priorities applicable to the region as outlined in the PSP?

   XYes **-**No

   How?

   Ecosystem Restoration Program Strategic Goals Mostly applicable to Goal 6, sediment and water quality. Also related to Goal 1, at-risk-species, and Goal 3, harvestabel species, because of the importance of chironomids in the salmon diet.

   Regional Implementation Priorities Related to MR-5, ensuring restoration work is not degraded by water quality. This information could also be useful in filling conceptual model gaps MR 6, SR 7.

   CVPIA Priorities Because this study involves a common prey item in the salmon diet, it could also qualify under the Anadromous Fish Restoration Program umbrella (3406(B)(1)).

3. Is the project adequately linked with other restoration activities in the region, such as ongoing implementation projects and regional planning efforts?

   XYes **-**No

How?

This work is planned to accompany another CALFED proposal addressing the same question but with a different, more time-tested method. This proposal also builds on recent CALFED funded work by UC Berkeley (Don Weston, approx. 50% complete) to test salmon prey items near agricultural runoff zones for toxins. Preliminary results from the UCB study triggered this proposal.

4. Does the project adequately involve local people and institutions?

XYes **-**No

How?

Working closely with the CALFED Toxicity of Unknown Cause Focus Group. This group also intends to involve local agencies and groups when soliciting for samples. Such groups include the Sacramento River Watershed Program, Grasslands Bypass Project, and Deltakeeper. They also plan on collaborating outreach efforts with SFEI.

Other Comments:

None

San Joaquin Regional Review:

Proposal Number: 221

Applicant Organization: US Army Corps of Engineers

Proposal Title: Identifying the cause of toxicity in environmental samples through gene expression fingerprinting

Overall Ranking: **X**Low **-**Medium **-**High

Provide a brief summary explanation of the committee's ranking:

This proposal takes a shot-gun approach to the search for the causes of unknown toxicity. The more experimental aspects of the investigation should be better understood and demonstrated before the approach is used to determine the causes of toxicity in the region.

1. Is the project feasible based on local constraints?

   -Yes **X**No

   How?

   The hypothesis upon which the proposal is based is interesting and the author has provided some background to establish the feasibility of the approach. The proposal to use an experimental method to search for the cause of unknown toxicity places too many variables in the way of results that might be usefully applied to the issues of concern in the region.

2. Does the project pursue the restoration priorities applicable to the region as outlined in the PSP?

   XYes **-**No

   How?

   In the most general way, the priority addressed is degradation of water quality by toxicity of unknown origin.

3. Is the project adequately linked with other restoration activities in the region, such as ongoing implementation projects and regional planning efforts?

   XYes **-**No

   How?

   The proposal offers to work with other groups to share samples, and offers to use the technique being developed on other sample sets.

4. Does the project adequately involve local people and institutions?

-Yes **X**No

How?

There is no connection in this proposal to any local people or institutions.

Other Comments:

The hypothesis is very interesting and may in the future yield a useful technique for assessing adverse biological effect. The connection to CalFed priorities is, however, tenuous at this point.

Sacramento Regional Review:

Proposal Number: 221

Applicant Organization: US Army Corps of Engineers

Proposal Title: Identifying the cause of toxicity in environmental samples through gene expression fingerprinting

Overall Ranking: **X**Low **-**Medium **-**High

Provide a brief summary explanation of the committee's ranking:

This is basic research on developing new test protocols for determining the sources of toxicity. ERP benefits, if any, will be a long time in coming.

1. Is the project feasible based on local constraints?

   XYes **-**No

   How?

   This appears to be 'cutting edge' technology, but study methods have been previously tried and have shown 'promising results'.

2. Does the project pursue the restoration priorities applicable to the region as outlined in the PSP?

   XYes **-**No

   How?

   Indirectly, through development of new protocols for identification of the source(s) of toxicity in aquatic environments.

3. Is the project adequately linked with other restoration activities in the region, such as ongoing implementation projects and regional planning efforts?

   XYes **-**No

   How?

   This is basic research not directly linked to any other planning or implementation efforts. It does attempt to address the issue of 'unknown toxicity' which commonly results in Bay/Delta biotoxicity studies.

4. Does the project adequately involve local people and institutions?

   XYes **-**No

How?

This is a research study and does not require major involvement of local communities and other institutions. Work would be done at Corps aquatic toxicity lab in Vicksburg MS

Other Comments:

This is basic research on developing new test protocols for determining the sources of toxicity. ERP benefits, if any, will be a long time in coming.

External Scientific: #1

Research and Restoration External Scientific Review Form

Proposal Number: **221**

Applicant Organization: **US Army Corps of Engineers**

Proposal Title: **Identifying the cause of toxicity in environmental samples through gene expression fingerprinting**

Conflict of Interest Statements:
I have no financial interest in this proposal.
XCorrect
-Incorrect

In the blank below please explain any connection to proposal, to applicant, co-applicant or subcontractor or to submitting institution (write "none" if no connection):

None

Review:

Please provide an overall evaluation summary rating:

<u>Excellent:</u> outstanding in all respects;
<u>Good:</u> quality but some deficiencies;
<u>Poor:</u> serious deficiencies.

Overall Evaluation Summary Rating	**Provide a brief explanation of your summary rating**
-Excellent	**The use of the microarray technique to identify sources of toxicity has great promise, and these investigators are capable of advancing the field. However, the proposal as written likely overextends its reach, due to difficulties in making the transition from acute laboratory exposures to complex lower level field exposures, and the budget as written does not provide enough detail to be easily evaluated.**
XGood	
-Poor	

1. **<u>Goals.</u>** Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the concept timely and important?

   Rating: Excellent

   The emerging technology of cDNA microarrays has shown promise in preliminary work in the field of human toxicology, and is anticipated to be of great value in environmental toxicology as well.

2. **<u>Justification.</u>** Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full-scale implementation project

justified?

Rating: GoodVery Good

Advances in the ability to identify toxic contaminants in the Delta and its tributaries will be valuable to resource managers in their efforts to control and remove sources of contamination.

With respect to identifying toxic contaminants, cDNA microarrays have numerous theoretical advantages over the currently used TIE method. From a technical view point, microarrays have greater specificity and sensitivity, are not limited by the environmental matrix to be studied, and are considerably less expensive per sample compared with the TIE method. In addition, unlike the TIE method, microarrays can provide insight into many other toxicological parameters. For example, they can be used to elucidate bioavailability, serve as biomarkers of exposure, document an organisms molecular response to contaminants, and elucidate a compounds mechanism of action. The development and use of microarrays therefore potentially offers a much wider range of scientific benefits than current methods.

The choice of the aquatic invertebrate C. tentans for this work is beneficial for a variety of reasons it is routinely used in standard EPA testing of toxic compounds, it can be used to assess toxicity of water or sediment, and it is the dominant prey organism for the juvenile fall-run chinook salmon in the lower Sacramento River. Also, the choice to study contaminants that are of primary concern in the Sacramento-San Joaquin watershed indicates an emphasis on environmental relevance.

3. **Approach.** Is the approach well designed and appropriate for meeting the objectives of the project? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology or approaches? Will the information ultimately be useful to decision-makers?

   Rating: Good

   The dose-response exposures of C. tentans are the first step in developing the microarrays, and only include acute toxicity at EC10 and EC50 concentrations. These concentrations are not environmentally relevant, which is especially important since microarrays generated under these exposure conditions will be compared with those generated by exposure to environmental samples. Because "the pattern of gene expression changes as concentration increases", the gene expression pattern (GEP) that can be elicited under acutely toxic laboratory conditions may not be similar to that elicited by more environmentally relevant, sublethal concentrations. This could make comparisons between the library of transcriptional responses and the GEP from environmental samples difficult. An addition to the proposal of the use of sublethal laboratory exposure concentrations therefore seems especially warranted. Also, since the environmental samples to be tested will include complex mixtures of contaminants, a validation of the microarray approach could be strengthened by the "testing" of spiked samples containing more than the proposed one to three compounds.

   With respect to testing of environmental samples, it would be helpful to include more information on exposure duration, choice of chironomid life stage, and which (sublethal) toxicological endpoints will be evaluated. Also, only five to eight environmental samples will be tested. Considering that the goal of the overall project is to identify toxic compounds in environmental samples, and that samples that have been toxicologically characterized are readily available, the number of actual samples to be tested seems surprisingly small.

4. **Feasibility.** Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives?

   Rating: Good

   C. tentans has been chosen for study in part because of its importance as a food organism for at-risk fish species. Although the proposed development of microarrays for C. tentans would help to identify which toxic contaminants an organism is responding to in an environmental sample, the use of microarrays in evaluating the potential adverse effects of these contaminants on the organisms health (and thus the availability of this organism as a food source for fish) is also very important. If sublethal toxicological endpoints, such as effects on growth or reproduction, could be evaluated during the laboratory dose-response exposures that are part of the microarray development process, then these adverse effects might be linked to the GEPs that are associated with different contaminants. In this way, the utility of the microarrays could extend from identification of contaminants in an environmental sample to an assessment of potential adverse health effects, especially ones that might not be phenotypically apparent during short term laboratory exposures. Although it is stated in the proposal that "The change in expression in individual genes and groups of genes will be correlated to whole animal toxicity measures (i.e. animals exposed to multiple toxicant levels below lethal concentrations)", the establishment of such a correlation, or the use of sublethal exposure concentrations, did not appear to be discussed further in any section of the proposal. A serious focus on this link between GEPs and adverse health effects would significantly extend and strengthen the usefulness of the microarrays, and in turn, the proposal itself. Focus on this link appears to be well within the scope of this project and the expertise of the researchers who will be involved.

   It is not clear whether the preliminary results obtained using restriction fragment differential display (RFDD) to assess gene expression in contaminant-exposed C. tentans would be predictive of success with the development of microarrays for this organism, especially since the methods employed for measuring gene expression in RFDD versus microarrays are very different. Also, construction of a subtracted library as the second step in developing a microarray involves the pooling of cDNAs from individual exposures to approximately eighteen different contaminants. A higher level of confidence in the potential success of the simultaneous development of microarrays for this many compounds would be achieved if prior success had been demonstrated in using a single compound to develop a microarray for this organism.

5. **Project-Specific Performance Measures.** Does the project include appropriate performance measures to measure success relative to the project's goals and objectives? Is there enough detail as to how the performance measures will be quantified? For restoration projects, are monitoring plans explicit and detailed enough to determine if performance measures will be adequately assessed?

   Rating: Very Good

6. **Products.** Are products of value likely from the project? Specifically for restoration projects, are products of value also likely from the monitoring component? Are interpretative outcomes likely from the project?

   Rating: GoodVery Good

   The fact that hundreds to thousands of genes will be cloned, sequenced, identified, and assessed for differential expression due to toxicant exposure means that much genetic information is likely to be generated which could help to provide insight into the underlying molecular mechanisms that govern responses to toxicant exposure. Although a thorough

evaluation of this type of genetic information is not within the scope of this proposal, this information (i.e. product) could be of value in future research that could focus on assessing mechanistic questions.

7. **Capabilities.** What is the track record of applicants in terms of past projects? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

   Rating: GoodVery Good

   The US Army Corps laboratory is well equipped with many (expensive) technological instruments that are essential for carrying out this project, including high through-put automated DNA sequencers, fluorescent imagers, high through-put thermocyclers for PCR, DNA microarray spotting robots, and microarray slide readers.

8. **Cost/Benefit Comments.** Is the budget reasonable and adequate for the work proposed?

   Rating: Good

   A more detailed break down of research expenses for different steps in the proposed work would have permitted a thorough assessment of the Budget Summary or Budget Justification.

Miscellaneous comments:

External Scientific: #2

Research and Restoration External Scientific Review Form

Proposal Number: **221**

Applicant Organization: **US Army Corps of Engineers**

Proposal Title: **Identifying the cause of toxicity in environmental samples through gene expression fingerprinting**

Conflict of Interest Statements:
I have no financial interest in this proposal.
XCorrect
-Incorrect

In the blank below please explain any connection to proposal, to applicant, co-applicant or subcontractor or to submitting institution (write "none" if no connection):

NONE

Review:

Please provide an overall evaluation summary rating:

Excellent: outstanding in all respects;
Good: quality but some deficiencies;
Poor: serious deficiencies.

Overall Evaluation Summary Rating	**Provide a brief explanation of your summary rating**
XExcellent	**This is an exciting and novel project that offers a promising alternative to traditional TIE techniques.**
-Good	
-Poor	

1. **Goals.** Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the concept timely and important?

   The overall goal of the project is to utilize the information contained in gene transcription patterns to identify specific stressors to which Chironomus tentans (model organism) is responding and to apply this capability to identifying unknown toxicants in environmental samples. This overall objective will be achieved by developing a Chironomus tentans cDNA microarray and evaluating the ability of the array to identify and discriminate among toxicants.

   The hypothesis for the work is that exposure of an organism to a toxic substance will evoke a compound-specific pattern of gene transcription as the organism responds to the toxicant by synthesizing certain proteins.

This application of molecular methods to addressing ecotoxicological problems is both timely and novel.

2. **Justification.** Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full-scale implementation project justified?

   Justification for the project is provided by evidence for problems with unexplained toxicity that could not be addressed (for one reason or another) with traditional TIE methods. The applicant also documents the disadvantages of current TIE approaches, including high cost, lack of methods for certain key chemicals (e.g., pyrethroids) and poor success with certain media (i.e., sediment). Very clear conceptual diagram.

3. **Approach.** Is the approach well designed and appropriate for meeting the objectives of the project? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology or approaches? Will the information ultimately be useful to decision-makers?

   The approach is very well described in this proposal. The methods to be used are relatively novel (particularly for this kind of application) but they seem a promising approach based on results for single chemical responses. If successful the project could lead to significant advances in identifying the causes of toxicity in complex mixtures.

4. **Feasibility.** Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives?

   The feasibility of the approach has been demonstrated for single chemical exposures. The only way to know if it will work in complex mixtures in a field setting will be to try it. The methods have been fully documented in the proposal and the scale seems fully justified. Though perhaps less of a sure thing than some of the other projects the potential benefits make it worth the extra risk.

5. **Project-Specific Performance Measures.** Does the project include appropriate performance measures to measure success relative to the project's goals and objectives? Is there enough detail as to how the performance measures will be quantified? For restoration projects, are monitoring plans explicit and detailed enough to determine if performance measures will be adequately assessed?

   A number of performance measures were indicated (p. 12) and they include presentations, peer-reviewed publications, newsletter articles. There is the expectation that early in year 3 of the project a microarray will be available for testing and the target is set to be successful identification of toxicity in at least 75% of samples.

6. **Products.** Are products of value likely from the project? Specifically for restoration projects, are products of value also likely from the monitoring component? Are interpretative outcomes likely from the project?

   The most important product will be a cDNA microarray that will be made available (presumably for free?) to resource agencies or other parties involved in water or sediment toxicity testing.

7. **Capabilities.** What is the track record of applicants in terms of past projects? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

   The qualifications of the PI and subcontractors are well suited to this project. The appropriate infrastructure is in place. It is also a strength that statisticians will be involved in the microarray interpretation work which can potentially be quite complex.

8. **Cost/Benefit Comments.** Is the budget reasonable and adequate for the work proposed?

   This 3 year project has a total budget of $1,093,337. There is one PI and two subcontractors.

Miscellaneous comments:

Potential point of concern - differences in tolerance between Chironomus and other toxicity test species could possibly lead to inability to identify toxicant (e.g., if a chemical is not toxic to Chironomus at the concentration present there may be not gene response, but the chemical could still cause mortality in other sensitive species). Not sure how important this could be. For many reasons I think Chironomus is an excellent choice as a test organism (both practical and in terms of its distribution and ecological relevance)

Need to consider what the implications for this project would be if the other TIE project is not funded.

Is this the kind of product one would patent? If so what are the implications?

External Scientific: #3

Research and Restoration External Scientific Review Form

Proposal Number: **221**

Applicant Organization: **US Army Corps of Engineers**

Proposal Title: **Identifying the cause of toxicity in environmental samples through gene expression fingerprinting**

Conflict of Interest Statements:
I have no financial interest in this proposal.
XCorrect
-Incorrect

In the blank below please explain any connection to proposal, to applicant, co-applicant or subcontractor or to submitting institution (write "none" if no connection):

The applicant and I share co-authorship on a paper that is in preparation, though we have not directly collaborated (work was done by my graduate student, who collaborated with the applicant)

Review:

Please provide an overall evaluation summary rating:

Excellent: outstanding in all respects;
Good: quality but some deficiencies;
Poor: serious deficiencies.

Overall Evaluation Summary Rating	**Provide a brief explanation of your summary rating**
-Excellent	**Overall, a (very) good project that could lead to an important and powerful tool for identifying the cause of toxicity in environmental samples. However, several aspects of the approach make it either difficult to evaluate the chance of success or reduce this chance of success. Limitations in the number of toxicants, interactions and concentrations included in the development of the microarray are unavoidable, but a modelling (e.g. power analysis or something of that nature) of the required library size would have reduced the uncertainties. And the evaluation step of the project appears to lack a thorough and unbiased analysis of the success of the methodology in performing TIEs.**
XGood	
-Poor	

1. **Goals.** Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the concept timely and important?

   The goals, objectives and hypotheses are generally clearly stated and internally consistent. Some of these are not totally complete. E.g. the possibility that some genes could be turned off (or specific proteins not produced) as a consequence of exposure is not mentioned in the

hypothesis statement on p. 2 and some other instances. However, it becomes clear from the methods section that this possibility will be investigated. The concept is timely and important. In theory, the use of gene expression fingerprinting could be an excellent tool for TEI (Toxicity Identification Evaluations), with several advantages over currently used methodologies (e.g. more powerful and matrix independent). The rapid recent advances in molecular genetics and bioinformatics have caused this approach to be potentially feasible at this point in time where it would not have been so as recent as five years ago. This field has advanced to the stage where we can now start evaluating the use of gene expression fingerprinting in environmental toxicology.

2. **Justification.** Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full-scale implementation project justified?

   The study is justified relative to existing knowledge. The time is right now for this study (due to recent advances in the field) and I am not aware of similar studies currently being conducted. A conceptual model is clearly stated, and is in principle fairly straightforward. Exposure to a specific contaminant will result in a probably unique change in gene expression in exposed organisms. Thus when a specific change in gene expression pattern is noted in an organisms affected by toxicants, in can be used to determine which contaminant(s) are responsible for the toxic effect. This project is a research project. At this point, available information indicates that gene expression fingerprinting could be a very powerful tool for TEI, but the feasibility of this approach has yet to be evaluated. A research project like the one proposed here is exactly what is needed at this point.

3. **Approach.** Is the approach well designed and appropriate for meeting the objectives of the project? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology or approaches? Will the information ultimately be useful to decision-makers?

   The approach is in general well designed and appropriate for meeting the project's objectives. The general approach is to first develop the microarray with Chironomus tentans genes differentially expressed during toxicant exposure and to then evaluate their use in identifying the toxicants in unknown samples with toxic effects. It is not certain that the details in the approach are sufficient for meeting the objectives. Approximately 21 toxicants will be used (at 2 concentrations) to create a library of gene transcription patterns, with various groups of contaminants presented. Also, some binary and tertiary mixtures will be used. While 21 toxicants might seem a lot (though possibly a reasonable starting point), this is of course only a drop in the bucket for the large number of chemicals that are in everyday use and potentially the cause of toxic effects in the bay area. With e.g. 21 toxicants, there will be more than 200 of just the binary mixtures; a total clearly beyond the scope of this project. The actual number of mixtures to be included is not specified. What is a reasonable sample size for a library of gene expression data sufficient for TIE? And the concentration-dependence of gene expression profiles means that while using 2 concentrations is a step in the right direction, responses at other concentrations could be missed. Preliminary work on concentration dependence is needed in order to assess how reasonable it is to use 2 contaminant levels. Also, the final evaluation will use 5-8 toxic samples and the success of the gene expression fingerprinting will be evaluated on the basis of this methodology's success (compared to traditional methods) in identifying the toxic compounds. This sample size for evaluation appears rather small, and it is not clear how success will be measured considering the the samples used are real-world samples for which the true toxic compounds are unknown. For example, if the gene technology indicates for a specific sample that cadmium is responsible for the toxic effect and the traditional TIE procedures indicate that DDT is the responsible factor, how will it be decided which is the true/correct answer? Or when one

method identifies a toxicant and the other does not? Can it then be assumed that the one identified is the correct one? Chemical analyses will provide some information, but are not sufficient as TIE. Results are likely to add to the base of knowledge. While there are significant limitations to the results (see above) they will add a significant contribution to the knowledge base in this field and will provide at least preliminary information for evaluating the use of gene expression fingerprinting in TIE. Novel information will be obtained about a new approach in TIE, and if the approach is found to be feasible, it will provide information that is very valuable to decision makers:"what toxicants in a water or sediment sample are responsible for the observed toxic effects".

4. **Feasibility.** Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives?

   The approach is fully documented and appears technically feasible. However, some limitations and problems with the details were already pointed out in the previous section. Also, it is acknowledged that the data analysis issue can be a major obstacle (e.g. "the hardest problem is to identify known chemicals by their transcriptional profiles in the presence of a background of other known and unknown profiles"), but it is not clear how these obstacles will be overcome (other than "with an alternation of supervised and unsupervised learning techniques"). What are the chances that this will be an unsurmountable obstacle? The issues raised here and in the previous sections make it difficult to assess the likelihood of success. What library size is reasonable for success? What sample size is reasonable for the evaluation step? How can the evaluation itself be correctly interpreted/scored? What is the chance that the data analysis does not run into major obstacles? The scale of the project is consistent with the objectives. The scale of the project is already considerable the way it is, but nevertheless possibly still too small for a proper evaluation of gene expression fingerprinting techniques for identifying the cause of toxicity in environmental samples.

5. **Project-Specific Performance Measures.** Does the project include appropriate performance measures to measure success relative to the project's goals and objectives? Is there enough detail as to how the performance measures will be quantified? For restoration projects, are monitoring plans explicit and detailed enough to determine if performance measures will be adequately assessed?

   The performance measures specified consist mainly of a listing of presentations and publications anticipated for this project. Also, microarray availability for testing samples is listed as a metric. While success can be reflected in publications and presentations, it appears that more direct measures of success could be used. For example, the success of the developed microarrays in identifying toxicants responsible for toxic effects of environmental samples. Is a 10% success rate considered a success for the overall project? Or maybe 50 or 90%? Or maybe a doubling of the success rate for traditional TIE methods?

6. **Products.** Are products of value likely from the project? Specifically for restoration projects, are products of value also likely from the monitoring component? Are interpretative outcomes likely from the project?

   If the project is successful, the product of the research is the specific microarray developed for the chironomid C. tentans. But it would also, and probably more importantly, be a demonstration that this approach (gene expression fingerprinting) can be used for identifying the cause of toxicity in environmental samples. These would be very valuable products.

7. **Capabilities.** What is the track record of applicants in terms of past projects? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

   The applicant has experience in related projects, and appears to have the capabilities to conduct the proposed project. Infrastructure (including the equipment needed for this research) appears to be available at the Army Corps of Engineers. The applicant will be collaborating with Donald Weston and Terry Speed at UC Berkeley. The expertise provided by Terry Speed (statistics/bioinformatics) will be essential for the rather demanding data analysis component of this project. Is is unclear what expertise will be provided by Donald Weston. He has extensive experience in assessing effects of contaminants on benthic invertebrates, but does not appear to have experience in molecular genetics.

8. **Cost/Benefit Comments.** Is the budget reasonable and adequate for the work proposed?

   The budget is not very transparent, making it difficult to evaluate how reasonable the budget is for the proposed work. Also, the division of tasks between Vicksburg and Berkeley is unclear. It appears that the graduate student and postdoc will be working at Vicksburg with salary support through Berkeley. There is a substantial subcontract for UC Berkeley ($731K-$991K; which is at least 70% of the total project costs!), which appears to include funds for Donald Weston (without this collaborator's role becoming clear) but no labor hours or salary funds are listed for Terry Speed (though this collaborators's contribution to the project appears very essential). Will Terry Speed have the time (if no salary is provided) to provide the considerable collaboration? Or will the data analyses be done by the "UCB Staff Res. Assoc."? Funds for the graduate student salary (at $3,373/mo.) seems excessive, and would furthermore elevate the student's salary above that of the postdoc (at $3,227/mo.) TIE testing and chemical analyses will be done by unspecified contract laboratories. Are the budgeted values for these activities realistic if the laboratories have not been identified? Also, it is mentioned that TIE tsting will be done in the parallel project if that one is also funded, which would negate the need for separate funds for this activity in the current proposal.

Miscellaneous comments:

(none)

Prior Performance/Next Phase Funding:

New Proposal Number: 221

New Proposal Title: Identifying the cause of toxicity in environmental samples through gene expression fingerprinting

1. Prior CALFED project numbers, titles, and programs: (*list only projects for which you are the contract manager*)

   99-N08, Assessment of pesticide effects on fish & their food resources in the Sacramento/San Joaquin Delta, UC Berkeley, Ecosystem Restoration

2. Prior CVPIA project numbers, titles, and programs: (*list only projects for which you are the contract manager*)

   N/A

3. Have negotiations about contracts or contact amendments with this applicant proceeded smoothly, without persistent difficulties related to standard contract terms and conditions?

   -Yes **X**No **-**N/A

   If no, please explain any difficulties:

   NFWF does not have any direct agreements with US Army Corps of Engineers. Current recipient for 99-N08 is UC Berkeley. UC Berkeley is listed as a subcontractor to this proposal.

4. Are the status, progress, and accomplishments of the applicant's current CALFED or CVPIA project(s) accurately stated?

   XYes **-**No **-**N/A

   If no, please explain any inaccuracies:

   Project 99-N08 is scheduled to be complete March 2003. The proposal contemplates a start date of July 2002, although applicant states that this date is flexible.

5. Is the applicant's progress towards these project(s)' milestones and outcomes to date satisfactory?

   XYes **-**No **-**N/A

   If no, please explain deficiencies:

   UC Berkeley's progress under 99-N08 has been satisfactory.

6. Is the applicant's reporting, records keeping, and financial management of these projects satisfactory?

XYes **-**No **-**N/A

If no, please explain deficiencies:

7. Will the project(s) be ready for next phase funding in 2002, based on its current progress and expenditure rates?

-Yes **X**No **-**N/A

If no, please explain:

The proposal is not a next phase effort of 99-N08.

Other Comments:

Environmental Compliance:

Proposal Number: 221

Applicant Organization: US Army Corps of Engineers

Proposal Title: Identifying the cause of toxicity in environmental samples through gene expression fingerprinting

1. Are the legal or regulatory issues that affect the proposal identified adequately in the proposal?

   XYes **-**No

   If no, please explain:

2. Does the project's timeline and budget reflect adequate planning to address legal and regulatory issues that affect the proposal?

   XYes **-**No

   If no, please explain:

3. Do the legal and regulatory issues that affect the proposal significantly impair the project's feasibility?

   -Yes **X**No

   If yes, please explain:

Other Comments:

Budget:

Proposal Number: 221

Applicant Organization: US Army Corps of Engineers

Proposal Title: Identifying the cause of toxicity in environmental samples through gene expression fingerprinting

1. Does the proposal include a detailed budget for each year of requested support?

   XYes **-**No

   If no, please explain:

2. Does the proposal include a detailed budget for each task identified?

   XYes **-**No

   If no, please explain:

3. Does the proposal clearly state the type of expenses encompassed in indirect rates or overhead costs?

   -Yes **X**No

   If no, please explain:

   Percentages are provided predicated on the funding source, however, there is no expense component detail. Verify that OH rate for UCB fluctuates with funding source, since all funds will flow through a federal applicant.

4. Are appropriate project management costs clearly identified?

   XYes **-**No

   If no, please explain:

5. Do the total funds requested (Form I, Question 17A) equal the combined total annual costs in the budget summary?

   XYes **-**No

   If no, please explain (for example, are costs to be reimbursed by cost share funds included in the budget summary).

6. Does the budget justification adequately explain major expenses?

   XYes **-**No

If no, please explain:

7. Are there other budget issues that warrant consideration?

   XYes **-**No

   If yes, please explain:

   Cost share of $300K/year is identified, however, the proposals does not indicate how these funds will be applied to project.

Other Comments: